Taking your breath away

Valorization

Social & economic relevance

Preterm birth is defined by the World Health Organization (WHO) as birth before 37 weeks of gestation. It can have a tremendous impact on the health and life of the infant after birth (1). Globally, around 15 million infants are born preterm, which is more than 1 out of 10 births (1). In The Netherlands, in 2015 7.1% of all births were preterm (2). In absolute numbers, this accounted for 12,070 children out of a total number of 169,267 infants (2). Although within The Netherlands these numbers show a decrease compared to the year of 2013, globally still almost 1 million children die every year due to preterm birth-related complications (1, 3). Prematurity remains the main cause of death in children younger than five years (1). Even when preterm infants survive, they often will face a lifetime with disabilities and health problems (1). Therefore, the social and economic impact of preterm birth-related complications on families, society and the health care system remains high (2, 4, 5). The psychosocial and emotional impact on the family, such as psychological distress and maternal depression, mainly depends on the severity of the infant's health condition (4, 6). The economic costs related to preterm birth are large and include acute neonatal intensive care, ongoing long-term health care needs and the requirement for special education services (4). In The Netherlands, on average, the total number of days spent in the neonatal intensive care unit (NICU) and the hospital varied between 11 days (for a child born between 34.0-36.6 weeks) and 62 days (for a child born between 24.0-25.6 weeks) per child within the year of 2015 (2). This indicates the inverse relation between the gestational age at birth and the intensity of required neonatal care (7). In addition, prematurely born children have a higher chance to be re-hospitalized (6, 8). Preterm birth-related costs added up to $26.2 billion in the United States in 2005; and the first-year medical costs for preterm infants ($32,325) were on average roughly ten times higher than the costs for term infants ($3,325) (4). Overall, these numbers indicate that preterm birth can be considered as a serious global health problem (1, 4).

Respiratory problems belong to the most common complications in preterm infants (9). The development of the lungs into healthy and functional organs is crucial for life after birth. As preterm infants are born before completion of the *in utero* stages of lung development, these infants have a higher risk to develop respiratory complications either directly after birth or at a later age (such as respiratory distress syndrome and wheezing disorders) (9-11). This is especially true when the preterm infant is additionally exposed to antenatal inflammation, like chorioamnionitis (9). Chorioamnionitis is an intra-uterine inflammation or infection of the chorion and the amnion (fetal membranes), and the placenta (12-14). Intra-uterine inflammation is

one of the most essential risk factors of preterm birth, as it is involved in up to 50% of the preterm births <28 weeks (12, 15). Prematurely born infants who were exposed to chorioamnionitis had an increased risk of asthma compared with gestational age-matched infants who were not exposed to chorioamnionitis, indicating the association between exposure to chorioamnionitis and childhood asthma (16). The highest risk of asthma (odds ratio [OR] 4.4; 95% CI: 2.2-8.7) and wheezing (OR 4.0; 95% CI: 2.0-8.0) was observed in former preterm infants who were exposed to chorioamnionitis and who were born at a lower gestational age (16, 17). In addition, antenatal infection or inflammation is associated with a higher risk for chronic lung disease if preterm infants are postnatally exposed to extended mechanical ventilation or sepsis (18). These findings suggest that the consequences of intra-uterine inflammation are not limited to life directly after birth and that they can persist into childhood.

Factors that have contributed to an increased survival rate of preterm infants are administration of antenatal corticosteroids to increase pulmonary maturation and advancements within mechanical ventilation techniques (5). To realize such new advances and to improve current medical technology and neonatal care, extensive research is essential and indispensable. As intra-uterine inflammation is such a critical risk factor for respiratory complications, this thesis focused on the effects of exposure to intra-uterine inflammation on the fetal lungs. Furthermore, in this thesis, the pulmonary effects of several therapies such as antenatal corticosteroids and interleukin-2 (IL-2) were evaluated and discussed within the setting of intra-uterine inflammation.

Novelty & future directions

Within this thesis, novel insights were presented. High-dose IL-2 therapy has already been approved by the US Food and Drug Administration as a treatment for metastatic melanoma and renal cell carcinoma (19). Nevertheless, as far as we know, we were the first to describe the clinical potential of prophylactic IL-2 treatment for the fetal lungs (**chapter 3**) and the gut (20) in an *in vivo* setting of intra-uterine inflammation. However, more research is required to investigate the use of IL-2 within this new indication of intra-uterine inflammation. Currently there are no adequate data available regarding the use of human recombinant IL-2 (Proleukin® [aldesleukin]) during pregnancy. No sufficient animal research was performed yet to assess the safety for the developing fetus, the course of gestation and the peri- and postnatal development (21, 22). Therefore, additional pre-clinical studies need to be conducted, to assess risks and benefits (23). Future pre-clinical studies will also reveal the effects of prophylactic IL-2 when chorioamnionitis is followed by a postnatal second hit (such

as mechanical ventilation or sepsis); and whether IL-2 can be used as a therapy *after* the induction of intra-uterine inflammation (instead of the prophylactic approach).

After sufficient and detailed information is known regarding animal fetal toxicity, safety and dosing of IL-2, pre-clinical studies can be followed by clinical studies. Clinical trials will be designed in compliance with Good Clinical Practice to test the fetal and maternal safety, the efficacy, the dosage and potential side-effects of IL-2 in humans (23).

This thesis also provides novel insights about the timing of exposure to intra-uterine inflammation. The data within this thesis clearly indicate that the timing of intra-uterine inflammation is crucial for the fetal pulmonary outcome. In **chapter 2** and in (24), it was shown that the order of exposure to intra-amniotic inflammation and antenatal corticosteroids influenced the pulmonary inflammatory response and the Wnt/β-catenin signaling pathway. Furthermore, the fetal pulmonary inflammatory response was shown to be time-dependent and gestation-dependent. It is suggested that for the fetal pulmonary outcome multiple factors are crucial: the gestational age at the onset of exposure to intra-uterine inflammation, the gestational age at preterm birth and the duration of the exposure. This further emphasizes the need for the development of reliable biomarkers to detect the presence of intra-uterine inflammation during pregnancy.

Potential target groups & activities

Out of a scientific point of view, the data described within this thesis are of interest to scientists and researchers who are working in the field of intra-uterine inflammation, preterm birth or pediatric pulmonology. In addition to this scientific audience, the findings within this thesis will be of value to clinicians who are specialized in neonatal and perinatal care or come into contact with intra-uterine inflammation during their daily work. The clinical target group therefore consists –without limitation- of: neonatologists, neonatal nurses, pediatricians and (pediatric) pulmonologists. Although this thesis focused on the fetal outcomes, instead of the maternal effects, after exposure to intra-uterine inflammation, the described findings might also be of interest for clinicians who are also involved in maternal medicine, such as obstetricians and gynecologists.

An important non-scientific target group consists of patient associations or parent groups. Parent groups are crucial in raising awareness of the health problem and in improving the quality of care (4). In Europe, the European Foundation for the Care of Newborn Infants (EFCNI) is a network and organization which represents the interests

of preterm and ill neonatal infants and their families. It combines the power of all stakeholders, such as parents, policy makers, healthcare professionals and scientists (25). By doing so, it has successfully increased awareness, political attention and policy change regarding preterm birth across Europe (4). In addition, by promoting research, the EFCNI gathers and multiplies information to improve the quality of care for (ill) neonates (25). As this thesis focuses on the effects of antenatal inflammation on fetal lung development, it can be of interest to organizations such as the EFCNI to help raise awareness of this specific health problem within our society. Hopefully, raising awareness will eventually contribute to the development of new therapeutic strategies for this very vulnerable patient group.

Finally, the data within this thesis can be of interest to the pharmaceutical industry, as therapeutic interventions and strategies were discussed within this thesis. While the use of corticosteroids within the field of perinatology is not new, we are the first to present the potential of prophylactic IL-2 therapy for the lungs in a translational setting of intra-uterine inflammation (**chapter 3**). The pharmaceutical industry could be of help and support to further investigate the potential of IL-2 within the context of antenatal inflammation.

To reach these target groups and to make individuals within and outside of the scientific community aware or our findings, we published our research data in peer-reviewed scientific journals relevant to our field of research, such as Pediatric Research (**chapter 2**) and the American Journal of Physiology Lung Cellular and Molecular Physiology (**chapter 3**). Furthermore, the findings described within this thesis were presented to (inter)national audiences via oral and poster presentations at a conference, symposium, science day or research meeting.

To summarize, the data reported within this thesis are scientifically, socially and economically relevant. Therefore, the content of this thesis can be of interest to target groups within and outside of the scientific and clinical community. Although the results described within this thesis were obtained using a translational pre-clinical model, these data contribute to our knowledge regarding intra-uterine inflammation and its pulmonary effects; and may eventually lead to the set-up of novel clinical studies and the discovery of new medical advancements. Only innovations within the field of gynecology, neonatology and perinatology will in time lead to a reduction in the occurrence and severity of intra-uterine inflammation and the pulmonary complications associated with this indication. Reducing the prevalence and severity will also lower health care costs and the burden on the infant and its family, ultimately leading to a reduction in the economic and social impact of intra-uterine inflammation and its associated pulmonary effects.

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